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Research report

Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons

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Abstract

The causes and effects of neuronal degeneration are of major interest to a wide variety of neuroscientists. Paralleling this growing interest is an increasing number of methods applicable to the detection of neuronal degeneration. The earliest methods employing aniline dyes were methodologically simple, but difficult to interpret due to a lack of staining specificity. In an attempt to circumvent this problem, numerous suppressed silver methods have been introduced. However, these methods are labor intensive, incompatible with most other histochemical procedures and notoriously capricious. In an attempt to develop a tracer with the methodological simplicity and reliability of conventional stains but with the specificity of an ideal suppressed silver preparation, the Fluoro-Jade dyes were developed. Fluoro-Jade C, like its predecessors, Fluoro-Jade and Fluoro-Jade B, was found to stain all degenerating neurons, regardless of specific insult or mechanism of cell death. Therefore, the patterns of neuronal degeneration seen following exposure to either the glutamate agonist, kainic acid, or the inhibitor of mitochondrial respiration, 3-NPA, were the same for all of the Fluoro-Jade dyes. However, there was a qualitative difference in the staining characteristics of the three fluorochromes. Specifically, Fluoro-Jade C exhibited the greatest signal to background ratio, as well as the highest resolution. This translates to a stain of maximal contrast and affinity for degenerating neurons. This makes it ideal for localizing not only degenerating nerve cell bodies, but also distal dendrites, axons and terminals. The dye is highly resistant to fading and is compatible with virtually all histological processing and staining protocols. Triple labeling was accomplished by staining degenerating neurons with Fluoro-Jade C, cell nuclei with DAPI and activated astrocytes with GFAP immunofluoresence.
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Theme: Techniques in neuroscience
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Keywords: Neuropathology; Neurotoxicology; Kainic acid; Apoptosis; Necrosis; Excitotoxicity; Hypoxia

1. Introduction

The detection of neuronal degeneration is important for a number of reasons. Unlike other cells in the adult body, dead neurons are not typically replaced by new neurons, so neuronal loss is of great consequence. It is therefore important to detect neuronal degeneration caused by chemical agents, physical insult, or disease. By doing so, the risks associated with exposure to neurotoxic chemicals including drugs, food contaminants or environmental hazards can be determined and their exposure avoided. The detection of neuronal degeneration is also important in the understanding of neurodegenerative diseases and in the evaluation of putative neuroprotective drugs.

Early studies relied on traditional detection methods such as Nissl or hematoxylin and eosin (H & E) stains in which neuronal degeneration was inferred either by morphological changes or by changes in staining intensity. Although degenerating neurons tend to be hypochromic when Nissl

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stained and hyperchromic when H & E stained, there is no staining specificity, as both viable and degenerating neurons are labeled. Furthermore it has been demonstrated [1,21] that H & E hyperchromia is not a reliable marker of neuronal degeneration. A significant advance was the development of suppressed silver stains [2,4,6,12] that selectively label degenerating neurons. Although superior to the more routine methods, the suppressed silver methods were less than ideal in several aspects. These limitations include a propensity to be capricious, labor intensive, time consuming and incompatible with most multiple labeling procedures. In addition, several reports [7,11] suggest that the presence of argyrophilic dark neurons simply reflect exposure to an insult that will ultimately result in neurons either dying or recovering.

A significant advance was the development of the Fluoro-Jade dyes which are fluorescent ligands for the detection and localization of degenerating neurons. Structurally, the three Fluoro-Jade dyes are related. The most fundamental of these fluorochromes is Fluoro-Jade [19], which is disodium 5′/6′ carboxyfluorescein. Fluoro-Jade B [18] is a mixture of the following three related fluorochromes: (1) trisodium 5-(6-hydroxy-3-oxo-3H-xanthen-9-yl) benzene, 1,2,4, tricarboxylic acid; (2) disodium 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)-5-(2,4-dihydroxybenzol) terephthalic acid; and (3) disodium 2,5-bis(6-hydroxy-3-oxo-3H-xanthen-9-yl) terephthalic acid [22]. Although the resolution of the exact chemical structure of Fluoro-Jade C is presently under investigation, preliminary mass spectroscopy data suggests that it is the sulfate ester of one of the aforementioned fluorescent components of Fluoro-Jade B.

The first of these fluorochromes, Fluoro-Jade [19], was initially shown to label neuronal degeneration following diverse insults including exposure to kainic acid, domoic acid, ibogaine, MK-801, 3-nitropropionic acid (3-NPA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and physical trauma. Subsequent studies employed this compound to localize neuronal degeneration following exposure to MPTP [5], methamphetamine [3,17] and D-fenfluramine [20]. An analogue of this compound, Fluoro-Jade B [18], was subsequently found to have an even greater affinity for kainic acid induced neuronal degeneration, as reflected by staining with increased contrast and resolution. Subsequent studies using Fluoro-Jade B characterized the temporal progression of kainic acid induced lesions [10], and demonstrated the patterns of degeneration resulting from exposure to aurothioglucose [15] and methylenedioxymethamphetamine (MDMA) [16]. Similarly, the present study demonstrates that the recently developed Fluoro-Jade C exhibits the greatest affinity for degenerating neurons and therefore results in staining with the highest resolution and contrast of the Fluoro-Jade dyes.

The present manuscript was actually the product of a preliminary structure–activity relationship study designed to allow inferences to be made concerning the structure of the endogenous neurodegeneration molecule. Inferences would be based on altered staining of degenerating neurons resulting from the addition of specific functional groups to the dye molecule. Thus, FJ-B variants with the addition of two 5 carbon alkane chains resulted in a fluorescent dye with no affinity for degenerating neurons, while the substitution of two additional hydroxyl groups produced a non-fluorescent compound. Increasing the dye's acidity by incorporating a sulfonate group resulted in a fluorescent compound of relatively low affinity for degenerating neurons, while the formation of a sulfate ester resulted in a fluorescent molecule with an extremely high affinity for degenerating neurons. Only this latter compound, introduced here as Fluoro-Jade C, was characterized further because of its practical histological applications.

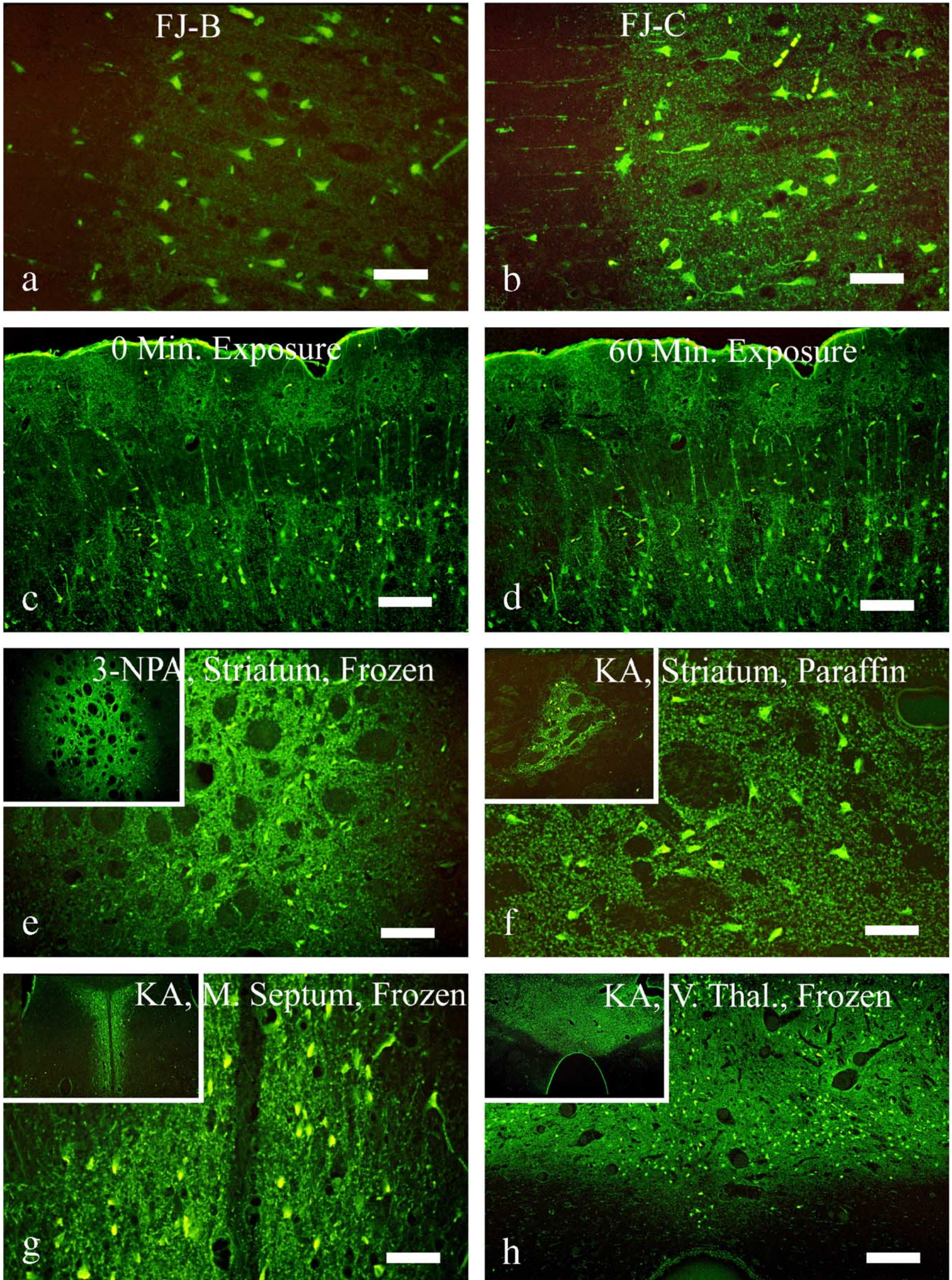
2. Materials and methods

2.1. Animal use

Ten adult male Sprague Dawley rats were given a single intraperitoneal injection of kainic acid (10 mg/kg), ten animals were given a single subcutaneous dose of 3-nitropropionic acid (30 mg/kg) and two animals were given a comparable volume of saline. All neurotoxicants were obtained from Sigma Chemicals (St. Louis, MO). All animals were used in accordance with institutional animal care and use guidelines. A survival interval of 2 days was used for animals receiving kainic acid, while a 4 day survival interval was used for animals receiving 3-nitropropionic acid (3-NPA). Animals were killed with an overdose of Nembutal (300 mg/kg) and then perfused transcardially with 400 ml of neutral phosphate buffered 10% formalin or 4% paraformaldehyde. Brains were removed and postfixed for at least 1 day in the same fixative solution. Those brains destined to be cut on a frozen microtome were postfixed in the fixative solution plus 20% sucrose.

2.2. Histochemical processing

Half of each group of brains were paraffin embedded and cut on a rotary microtome while the remainder were cut on a freezing sliding microtome. Paraffin sections were 10 μm in thickness while frozen sections were cut at a thickness of 25 μm. Prior to staining, sections were mounted from distilled water onto gelled slides. Gelatin coated slides were prepared by immersion in a 60 °C solution of 1% pig skin gelatin [Sigma; type A, 300 Bloom] and then oven dried overnight at the same temperature. The sections were mounted onto the slides from distilled water and then air dried for at least 30 min on a slide warmer at 50 °C. Slides bearing frozen cut tissue sections were first immersed in a basic alcohol solution consisting of 1% sodium hydroxide in 80% ethanol for 5 min. They were



FJ-B
a
FJ-C
b
0 Min. Exposure
c
60 Min. Exposure
d
3-NPA, Striatum, Frozen
e
KA, Striatum, Paraffin
f
KA, M. Septum, Frozen
g
KA, V. Thal., Frozen
h

then rinsed for 2 min in 70% ethanol, for 2 min in distilled water, and then incubated in 0.06% potassium permanganate solution for 10 min. Slides were then transferred for 10 min to a 0.0001% solution of Fluoro-Jade C (Histo-Chem Inc.; Jefferson, AR) dissolved in 0.1% acetic acid vehicle. The proper dilution was accomplished by first making a 0.01% stock solution of the dye in distilled water and then adding 1 ml of the stock solution to 99 ml of 0.1% acetic acid vehicle. The working solution was used within 2 h of preparation. The stock solution, when refrigerated, can be kept for long periods but should be discarded if the solution becomes cloudy. The slides were then rinsed through three changes of distilled water for 1 min per change. Excess water was drained onto a paper towel, and the slides were then air dried on a slide warmer at 50 °C for at least 5 min. The air dried slides were then cleared in xylene for at least 1 min and then coverslipped with DPX (Fluka or Sigma) nonfluorescent mounting media. Polar coverslipping media, such as those that contain water, alcohol or glycerol were never used.

For comparative purposes, some slides were stained with Fluoro-Jade B according to the previously described procedure [18]. When working with paraffin processed tissue, the sections are first deparaffinized through two 10 min changes of xylene and then the sections are rehydrated through a graduated alcohol series, omitting the basic alcohol solution. Once in distilled water, the sections are transferred to the potassium permanganate solution at which point the staining procedure is identical to that described for frozen sections.

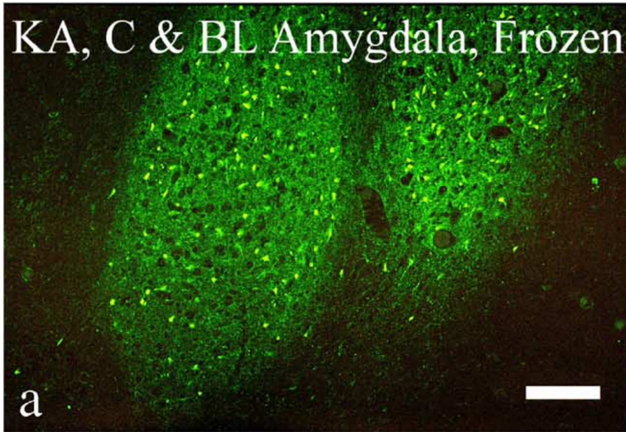
2.3. Multiple labeling

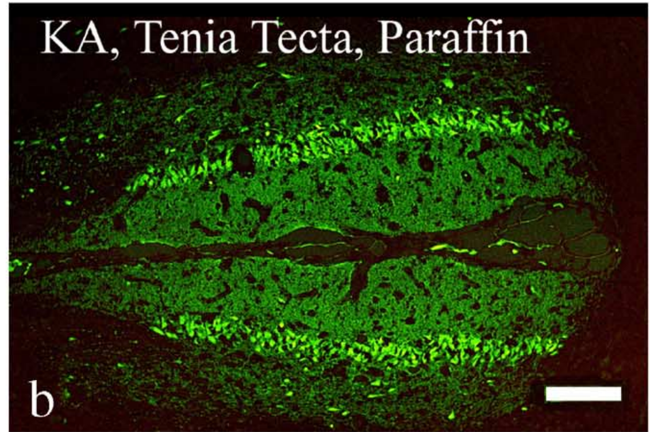
Fluoro-Jade C can readily be combined with other fluorescent markers. In this study, multiple labeling was achieved using anti-glial fibrillary acidic protein (GFAP; DiaSorin, Stillwater MN) immunocytochemistry to label activated astrocytes while using DAPI to label nuclear DNA. Incorporating 4′, 6-diamidino-2-phenylindole (DAPI; Sigma, St. Louis MO) as a fluorescent nuclear stain is accomplished by simply incorporating 0.0001% into the Fluoro-Jade C staining solution. This is accomplished by the addition of 1 ml of 0.01% DAPI stock solution to 99 ml of 0.1% acetic acid. Fluoro-Jade C was also combined with immunofluorescent labeling of GFAP according to the following procedure. Loose frozen tissue sections were incubated in a prediluted solution of anti-GFAP at about 5 °C in the refrigerator for 1–3 days. It should be mentioned that although in this study all immunocytochemistry was performed on frozen sections, the methods are fully compatible with paraffin processed tissue as well. Sections were rinsed in two changes of buffered saline for 10 min each and then transferred to a tetramethylrhodamine isothiocynate (TRITC) labeled secondary antibody (Chemicon, Temecula CA), diluted 1:100 in buffered saline, for 1 h at room temperature. Sections were rinsed in two changes of buffered saline for 10 min each and then the sections were mounted onto gelled slides from distilled water and air dried on a slide warmer at 50 °C for 30 min. To combine with Fluoro-Jade C, the slide mounted sections were rehydrated for 2 min in distilled water and then transferred to the 0.06% potassium permanganate solution for 10 min. It is worth mentioning that the incubation time in potassium permanganate may need to be reduced when co-localizing those antigenic epitopes susceptible to chemical oxidation. The slides were then rinsed for 2 min in distilled water, transferred to the Fluoro-Jade C working solution for 10 min and then rinsed, air dehydrated, xylene cleared and coverslipped with DPX, as previously described. The blue nuclear label conferred by DAPI is visualized via ultraviolet light excitation, while the red TRITC labeled antibody is visualized by green light excitation.

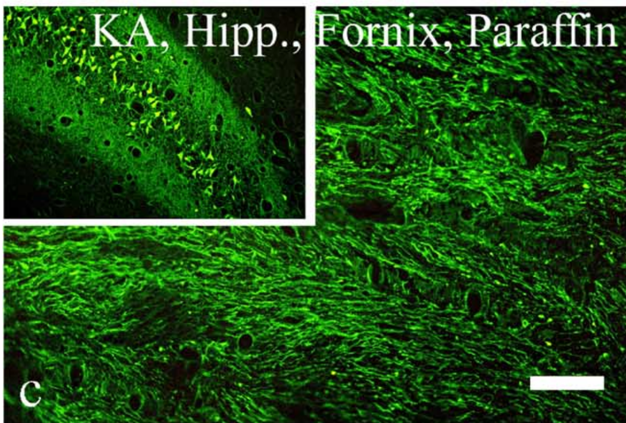
2.4. Analysis

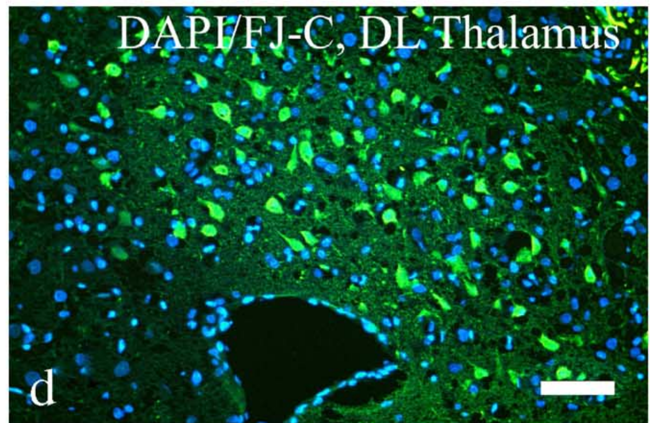
The Fluoro-Jade C labeled sections were examined under an epifluorescent microscope using a filter system designed for visualizing fluorescein (Ex: 385 nm, Em: 425 nm) or fluorescein isothiocynate (FITC). Photographic documentation of multiple labeling was accomplished by sequential multiple exposures combining different excitation wavelengths (i.e. ultraviolet, blue and green light). To evaluate Fluoro-Jade C for perma-

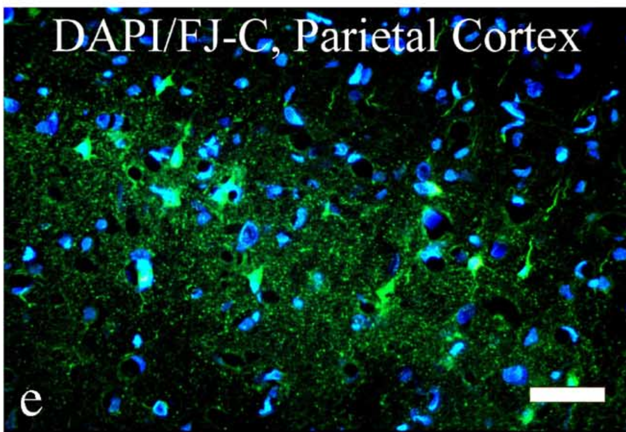
Fig. 1. (a and b) Comparison of kainic acid induced neuronal degeneration in adjacent cingulate cortex sections stained with Fluoro-Jade B (a) vs. Fluoro-Jade C (b). Note that Fluoro-Jade C results in higher resolution and contrast staining, especially of the small degenerating axon terminals. Mag. bars = 40 μm. (c and d) Comparison of the fading seen in the same tissue section, from the kainic acid-treated cingulate cortex stained with Fluoro-Jade C, immediately after preparation (c) vs. after 60 min of epifluorescent illumination via a 20× objective (d). Note that the fading is negligible. Mag. bars = 100 μm; figures rotated 90° clockwise. (e) Illustration of the striatal lesion produced by 3-NPA exposure. Insert is low magnification (4×) survey view. This toxicant results in a focal lesion in which degenerating soma, neuropil and terminals are all Fluoro-Jade C positive, while the intact surrounding tissue, the myelinated fascicles and the blood vessels are all negative. Mag. bar = 100 μm. (f) Illustration of the kainic acid induced degeneration in paraffin sections through the striatum. Low magnification (4×) insert shows localized patch of degenerating neurons in the lateral striatum. High magnification view reveals conspicuously labeled cell bodies and distinct terminals. Mag. bar = 40 μm. (g) Illustration of kainic acid induced degeneration in the medial septum. Low magnification insert (4×) of the frozen tissue section reveals the overall pattern of degeneration, while a higher magnification shows the presence of degenerating cells and numerous terminals. Mag. bar = 40 μm. (h) Reveals extensive kainic acid induced lesion of the ventral thalamus and zona incerta seen in insert at low magnification (4×). In contrast, virtually no damage is seen in the underlying hypothalamus. Higher magnification reveals the presence of degenerating cells and terminals. Mag. bar = 250 μm.


KA, C & BL Amygdala, Frozen
a


KA, Tenia Tecta, Paraffin
b


KA, Hipp., Fornix, Paraffin
c


DAPI/FJ-C, DL Thalamus
d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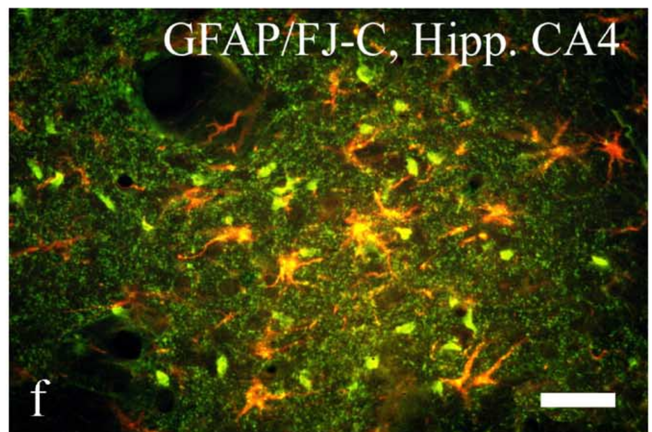
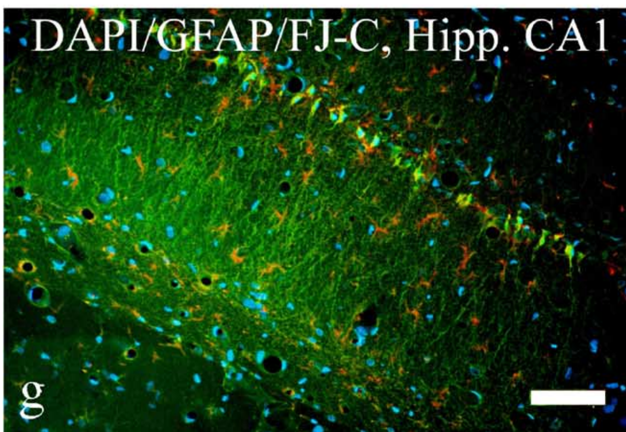
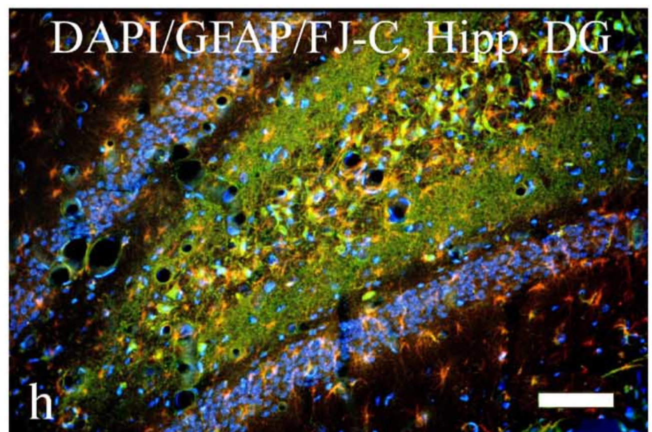
e


GFAP/FJ-C, Hipp. CA4
f


DAPI/GFAP/FJ-C, Hipp. CA1
g


DAPI/GFAP/FJ-C, Hipp. DG
h

nence, both 1 year old archival tissue and tissue exposed to prolonged epifluorescent illumination were evaluated. The latter was accomplished by photographing stained tissue with a 20× objective and then re-photographing the same tissue after 60 min of intense (100 W, new Hg bulb) epi-illumination.

3. Results

3.1. Saline treated control tissue

Labeling of neurons in control tissue was nonexistent. In those brains that were incompletely perfused, stained red blood cells could be observed within vascular elements. Some tissues adjacent to the brain also stained, specifically the meninges and the choroid plexus. Although glial labeling is not common, occasionally weakly stained astrocytes could be observed within certain white matter structures such as the middle cerebellar peduncle.

3.2. Comparing FJ-B vs. FJ-C labeling

Neurons were labeled in their entirety including cell bodies, dendrites, axons and terminals. Definition of fine cellular processes, including distal dendrites, axons and terminals was sharp (Fig. 1b). In contrast, labeling of these fine structures with Fluoro-Jade (not illustrated) or Fluoro-Jade B (Fig. 1a) resulted in a more diffuse, lower resolution stain. The Fluoro-Jade C staining also exhibited the highest contrast with brightly labeled neurons fluorescing against an almost black background.

3.3. Tissue permanence

Examination of archival tissue stained the previous year did not reveal any detectable reduction in signal strength or loss of resolution (data not shown). When the same stained cells were exposed to 0 versus 60 min of high intensity continuous epi-fluorescent illumination, little difference was observed (Figs. 1c, d). However, the photographic exposure time was approximately double after an hour of continuous illumination, and subsequent low magnification examination of the area revealed a darker circular area where the background staining had been bleached by prolonged high magnification examination.

3.4. Sectioning methods

The 25 um thick frozen sections (e.g., Figs. 1e and g) were useful for revealing neuronal clusters or nuclei as well as cellular cytoarchitectonics. The 10 μm thick paraffin embedded tissue sections (e.g. Figs. 1f, 2b and e) were best for resolving fine neuronal processes such as axons and terminals.

3.5. Kainic acid treated tissue

The pattern of neuronal degeneration resulting from kainic acid exposure is consistent with previous reports [13,10]. Degeneration was observed throughout the forebrain. The most extensive degeneration was seen in the hippocampus (Figs. 2c, g, h), piriform cortex, ventral thalamus (Fig. 1h), medial thalamus (Fig. 2d), septum (Fig. 1g), tenea tecta (Fig. 2b), and amydgala (Figs. 2a, f). Degeneration was sporadic in neocortical regions (Fig. 2e) and in the lateral striatum (Fig. 1f). In some regions of extensive neuronal degeneration, such as the hippocampus, hypertrophied astrocytic profiles were occasionally observed, although less intensely stained than adjacent degenerating neurons.

3.6. 3-Nitropropionic acid treated tissue

The pattern of degeneration resulting from 3-nitropropionic acid exposure is consistent with previous reports [9]. The most consistent and extensive damage was to the striatum (Fig. 1e). This lesion is typically bilateral and is characterized by staining of the surrounding neuropil and cell bodies, but not the penetrating myelinated fascicles. In two cases, the lesion also included the ventral thalamus, hippocampus, or deep nuclei of the cerebellum. Astrocytic staining was not observed.

Fig. 2. (a) Demonstrates extensive kainic acid induced degeneration in both the basolateral amygdala (left) and the dorsal endopiriform nucleus (right). Mag. bar = 100 μm. (b) Illustrates extensive kainic acid induced degeneration of the tenia tecta. Mag. bar = 100 μm, image rotated 90° counterclockwise. (c) Examination of the fornix following exposure to kainic acid reveals extensive axonal labeling. Fluoro-Jade C positive hippocampal cells of origin are seen in the insert (4×). Mag. bar = 40 μm. (d) Double exposure using ultraviolet and blue light excitation reveals blue DAPI labeled nuclei and green Fluoro-Jade C positive cells and terminals in the dorsal thalamus following kainic acid exposure. Mag. = 40 μm. (e) Double exposure using blue and ultraviolet light excitation results in the respective localization of green colored Fluoro-Jade C positive degenerating neurons and blue colored cell nuclei in layer VI of the parietal cortex following kainic acid exposure. Note conspicuous Fluoro-Jade C staining of terminals as well as cell bodies. Mag. bar = 40 μm. (f) High magnification of the central nucleus of the amygdala is visualized by using sequential green and blue illumination to reveal red GFAP labeled activated astrocytes in association with green Fluoro-Jade C positive degenerating nerve cells and terminals. Mag. bar = 40 μm. (g) Triple labeling in the hippocampus following kainic acid exposure is accomplished by sequential multiple exposures using ultraviolet, blue and green light excitation. Fluoro-Jade C positive CA1 pyramidal cells and their dendrites appear green, GFAP positive astrocytes appear red, and DAPI positive cell nuclei appear blue. Mag. bar = 100 μm. (h) A triple exposure of the hippocampal dentate gyrus sequentially illuminated with green, blue and ultraviolet light reveals Fluoro-Jade C positive degenerating polymorphic neurons in the hilar region, DAPI staining of nuclei which is especially conspicuous among the intact granule cells and GFAP positive activated astrocytes. Mag. bar = 100 μm.

3.7. Multiple labeled tissue

Sections co-stained with DAPI resulted in a blue fluorescence of cellular nuclei (Figs. 2d, e). Such nuclear staining was seen in all viable cells. Although some Fluoro-Jade C positive cells were double labeled with DAPI, other degenerating neurons did not exhibit obvious nuclear staining. Those sections with TRITC labeled anti-GFAP exhibited red appearing astrocytes. Those astrocytes adjacent to Fluoro-Jade C positive neurons were typically hypertrophied and brightly stained (Fig. 2f). When both co-labels are combined with Fluoro-Jade C, the predictable expression of green degenerating neurons, blue nuclei, and red astrocytes were seen simultaneously (Figs. 2g, h).

4. Discussion

The findings of this study indicate that Fluoro-Jade C is the most sensitive of the fluorescent markers of neuronal degeneration in terms of producing a stain of highest resolution and contrast. This allows for the unequivocal localization of the finest nerve processes, including distal dendrites, axons and terminals. The ability to label with increased resolution implies that Fluoro-Jade C possesses a higher affinity for the endogenous neurodegeneration molecule(s) than its predecessors Fluoro-Jade or Fluoro-Jade B. It also is used at the lowest concentration and requires the shortest staining time. This might seem paradoxical, considering preliminary spectrofluorometric data indicate that Fluoro-Jade C is the least fluorescent of the three related fluorochromes. Presumably the lower fluorescence is more than compensated for by a higher affinity for degenerating neurons. The histological parameters described here were designed to result in minimal background staining. Certain special circumstances such as using unfixed tissue, prolonged rinsing, or using a microscope with less than optimal fluorescence optics may require increased (e.g. 2–4 fold) dye concentrations.

Despite the fact that there is considerable information known about the structures of the Fluoro-Jade dyes [22], considerably less is known about the chemical identity of the endogenous degeneration molecule to which it binds. As all of the Fluoro-Jade dyes share the property of possessing multiple acidic substitutions, we have previously speculated [18] that the degeneration molecule is a polyamine. Consistent with this theory, a number of polyamines are associated with cellular degeneration including putrescine, cadaverine, spermadine and histamine. However, there is no evidence that these isolated polyamines are unique to degenerating neurons, or that they would not evaporate or dissolve during histological processing. Therefore, the expression of polyamines via cleavage of a larger biomolecule might be a more plausible theory. The morphological location of this putative bio-molecule can be narrowed down to either the plasma membrane or cytoplasm, since the nucleus and various cellular organelles have no affinity for the dye. Consistent with a membrane based theory, a basic charged membrane component molecule, such as phosphotidyl choline, may shift its charge distribution upon neuronal cell death, as has been reported for phosphotidyl serine [14]. A cytoplasmic based theory would presumably involve a change, possibly decarboxylation, dephosphorylation or proteolytic cleavage of a neuron specific cytoplasmic molecule such as those associated with the neurofilaments or microtubules. A cleaved microtubule protein, c-tau, has been reported to be expressed by degenerating neurons following a number of insults including exposure to kainic acid [23]. It is also not known if the endogenous degeneration molecule is produced via an anabolic or catabolic mechanism. An important follow-up to the present study will be the identification of the neurodegeneration molecule(s). It may be possible to accomplish this by the use of either 2D-Gel or affinity chromatography methods.

The neuronal labeling with Fluoro-Jade C is extremely specific for degenerating neurons. The relatively rare treatment-related staining of astrocytes appears to reflect astrocytic activation, as indicated by increased GFAP expression, rather than overt astrocytic death. Some cells outside the brain parenchyma such as red blood cells, choroid plexus and collagen fibrils of the meninges also stain in control tissue. Although vascular perfusion typically removes the red blood cells, it is possible to prevent these cells from labeling in nonperfused tissue by staining for peroxidase/catalyse enzyme activity using 3,3′diaminobenzidine HCl/H_2O_2 histochemistry [8], which will mask any red blood cells present (not illustrated).

Some fluorescent methodologies are hampered by the rapid fading of the fluorochrome. This is not typically a problem with Fluoro-Jade C, which is highly photostable, especially when used in conjunction with the potassium permanganate pretreatment. No loss of signal could be detected in year old archival tissue. While some fading could be detected after a full hour of intense epi-illumination, it was minimal and did not hamper analysis and photographic documentation, as illustrated (Figs. 1d, e). However, as prolonged illumination did result in detectable fading of the background, low magnification views are best photographed before extended examination at higher magnification.

The Fluoro-Jade C staining technique is generally flexible in terms of the types of tissue processing with which it is compatible and is quite forgiving in terms of not requiring precise reagent concentrations and incubation times. Although the dye is compatible with tissues that are either fixed or unfixed, we typically use formalin fixed tissue as this results in a brighter stain of higher resolution. We also usually cut non-embedded sections on a freezing sliding microtome, while paraffin embedded sections are cut on a rotary microtome. Vibratome and cryostat cut sections

are less frequently used since, in our laboratory, the former has a tendency to produce sections with chatter, while the latter has a propensity to result in sections with freezing artifact. Although the staining procedure is relatively simple and straightforward, a few potential pitfalls should be avoided. For example, the slides must be properly gelatin coated, as described in the Material and methods section, to prevent section loss during processing. Also, after staining it is important to avoid the use of alcohol dehydration, or the use of a coverslipping media that contains polar solvents such as water, glycerin, or alcohol.

In conclusion, this study suggests that Fluoro-Jade C is the tracer of choice for localizing neuronal degeneration. It is superior to standard histological stains (e.g. Nissl and H & E) as it stains only degenerating neurons and is not prone to producing false positives. It also avoids limitations associated with suppressed silver methods such as being labor intensive, capricious, prone to generating false positives, requiring long post-fixation intervals, requiring the use of toxic reagents, and a general incompatibility with other histochemical techniques. Although the fluorescent predecessors to Fluoro-Jade C, Fluoro-Jade and Fluoro-Jade B, will reveal a similar pattern of neuronal degeneration the resolution and contrast will be lower. Thus, Fluoro-Jade is useful for detecting degenerating neuronal cell bodies, but lacks the resolution necessary for the unequivocal detection of fine degenerating distal dendrites, axons, or terminals. Fluoro-Jade B can detect these finer structures, although the resolution and contrast are noticeably less than seen with Fluoro-Jade C. In addition to increased contrast and resolution, Fluoro-Jade C is also preferable in that it requires the shortest processing time and the lowest dye concentration. This supports the notion that Fluoro-Jade C possesses the greatest affinity for the endogenous neurodegeneration molecule.

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